

Microbial Biomass in the Euphotic Zone of the North Pacific Subarctic Water¹

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ABSTRACT: Microbiological investigations were made in the North Pacific subarctic water during spring of 1969. Total bacteria, heterotrophic bacteria, yeasts, and glucose uptake by microorganisms were measured in the euphotic zone. There was no heterogeneity in the distribution of microbial biomasses between Alaskan Gyre, mid-Pacific transitional water, and Western Gyre. Another statistical analysis showed that there was microzonation in the microbial distribution but no specific vertical distribution of total bacteria, heterotrophic bacteria, and glucose uptake. The microbial biomass in the euphotic zone of the North Pacific subarctic water in spring was estimated to be $(3.0 \pm 1.4) \times 10^8$ clumps of total bacteria per ml, 2.1 ± 1.9 clumps of heterotrophic bacteria per 10 ml, and a glucose-carbon uptake of 89 ± 32 μg per m^3 per day.

THE STUDY reported here of bacterial activity over 10,000 miles of the subarctic North Pacific Ocean was carried out in conjunction with studies by other personnel on the primary, secondary, and tertiary production of the area. In this respect the particular purpose of the bacterial sampling was to determine the extent of changes with geographic area in the standing stock of bacteria and the rate of their production throughout the area.

Using data available up until 1946, ZoBell (1946) concluded that bacteria are not sufficiently abundant in seawaters to constitute an appreciable item in the diet of marine animals. However, he also stated that cumulatively bacteria must play an important role in food cycles by synthesizing cell substances and by converting waste or dissolved organic matter into particulate form which can be utilized as food by animals. In the early literature it was generally accepted that only the living organisms were important as food for particulate feeders in the ocean.

Using the data obtained at Station "P" in the Pacific Ocean, McAllister, Parsons, and Strickland (1960) and Parsons and Strickland (1962) reported that detritus accounts for the largest fraction of particulate matter in the sea, and

they stressed the importance of detritus in marine ecology. This finding was extremely important in considering the role of bacteria as food for marine animals, because on such detritus bacteria and allied microorganisms can exist and grow. With the theory of the function of solid surfaces as adsorbants of dissolved organic matter (ZoBell, 1946), the finding strongly supported the belief that bacteria and other heterotrophs are important in the food chain of the sea.

As part of a series of biological, physical, and chemical investigations of the Strait of Georgia, Seki and Kennedy (1969) studied the role of bacteria and concluded that bacteria and allied microorganisms are important as food for copepods, and that they have nearly the same quantitative importance as phytoplankton, especially in the euphotic zone during winter when primary productivity is low.

The present work is part of an oceanographic investigation to study the food chain in the subarctic Pacific Ocean carried out on board the research vessel CNAV "Endeavour" during a trans-Pacific crossing from March to May 1969 (Parsons, 1969).

MATERIALS AND METHODS

The trans-Pacific crossing followed an approximate great circle route from Esquimalt, British Columbia, to Tokyo, and back from

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Hakodate to Esquimalt. The distribution of water masses was determined on the basis of data on physical, chemical, and biological observations, and on the basis of microbiological data obtained at the stations in the water masses of the Alaskan Gyre (Stations 03 to 06, and 34 to 37), mid-Pacific Canal Transitional Water (Stations 07 to 10, and 30 to 33), and Western Gyre (Stations 11 to 14, and 26 to 29). The positions of the sampling stations are shown in Figure 1.

All seawater samples were collected with Cobet sterile water samplers at local apparent noon from depths of 2, 5, 10, 15, and 25 meters. The microbiological treatment was finished within half an hour of the collection of samples. For direct bacterial counts, 100 ml of each seawater sample were passed through a sterile HA Millipore filter. The filter was fixed in formalin vapor and stained with erythrosin. The number of microbial clumps (hereafter referred to as total bacteria) was calculated from microscopic counts.

Determination of viable organisms was made by filtering 10- and 100-ml portions of each seawater sample through sterile HA Millipore filters. The filters for 10-ml samples were placed on nutrient agar Medium 2216 (ZoBell, 1941) for the enumeration of heterotrophic bacteria, and those for the 100-ml samples on van Uden's Medium (van Uden and Castelo-Branco, 1961) for the enumeration of yeasts. All cultures were incubated for 20 days at 10°C before colonies were counted.

Counts for the total bacteria, heterotrophic bacteria, and yeasts were expressed as clumps

per unit volume of seawater, because they existed as aggregates in nature, and also because counting individual cells is impossible (Seki, 1968, 1969).

Glucose uptake by microorganisms in seawater samples was measured by the method of Parsons and Strickland (1962). After the addition of 250 mg of carbon as D-glucose per m³ of water, 2 μ Ci of uniformly labeled ¹⁴C-D-glucose was added to each sample of 100 ml. These were then incubated for 4 hours at *in situ* temperatures. The radioactivity taken up by the microorganisms was determined in a liquid scintillation counter (Nuclear Chicago Company) after filtration on HA Millipore filters.

RESULTS

The microbial biomass in the euphotic zone of the Alaskan Gyre, mid-Pacific Transitional Water, and the Western Gyre was analyzed statistically. No significant difference was found in the distribution of biomass or microzonation between the three areas. The F-test on the distribution of heterotrophic bacteria is shown in Table 1. Similarly, $F = 7.2$ was calculated for total bacteria, $F = 2.3$ for yeasts, and $F = 10.9$ for glucose uptake. All data together gave $F_{0.05} = 19.5$. For this reason, all the data from each microbiological investigation in the three water masses (Alaskan Gyre, mid-Pacific Transitional Water, and Western Gyre) were treated collectively and put into one class for later analyses.

Figure 2 shows the vertical distribution and activity of microorganisms at each depth. The

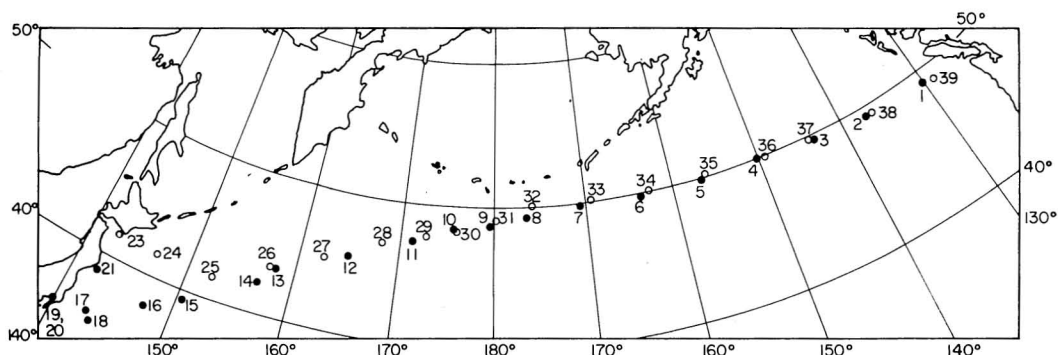


FIG. 1. Locations of the stations sampled for investigation. Stations 01 to 17 were taken during the trip from Canada to Japan; Stations 24 to 39 were taken from Japan to Canada.

TABLE 1
ANALYSIS OF VARIANCE IN THE DISTRIBUTION OF
HETEROTROPHIC BACTERIA IN THREE WATER
MASSES (ALASKAN GYRE, MID-PACIFIC
TRANSITIONAL WATER, AND WESTERN
GYRE) OF THE NORTH PACIFIC OCEAN

SOURCE OF VARIATION	SUM OF SQUARES	DEGREE OF FREEDOM	MEAN SQUARE	VARIANCE RATIO
S_b (between)	11	2	5.5	1.4
S_w (within)	337	85	4.0	
Total	348	87		

$F(2, 85; 0.05) = 3.1$

F-test shows that there was no significant difference between each depth except in the abundance of yeasts: $F = 1.6$ for total bacteria, $F = 4.1$ for heterotrophic bacteria, $F = 2.3$ for glucose uptake, and $F_{0.05} = 5.7$ for all. This means that the patchiness of microorganisms was independent of the vertical depth down to 25 meters below the surface. Only yeasts showed significant fluctuation in abundance ($F = 3.0^*$, and $F_{0.05} = 2.6$), having a larger biomass at 2 meters than at greater depths.

Frequency distribution of bacteria and microbial activity in the subarctic water are shown in

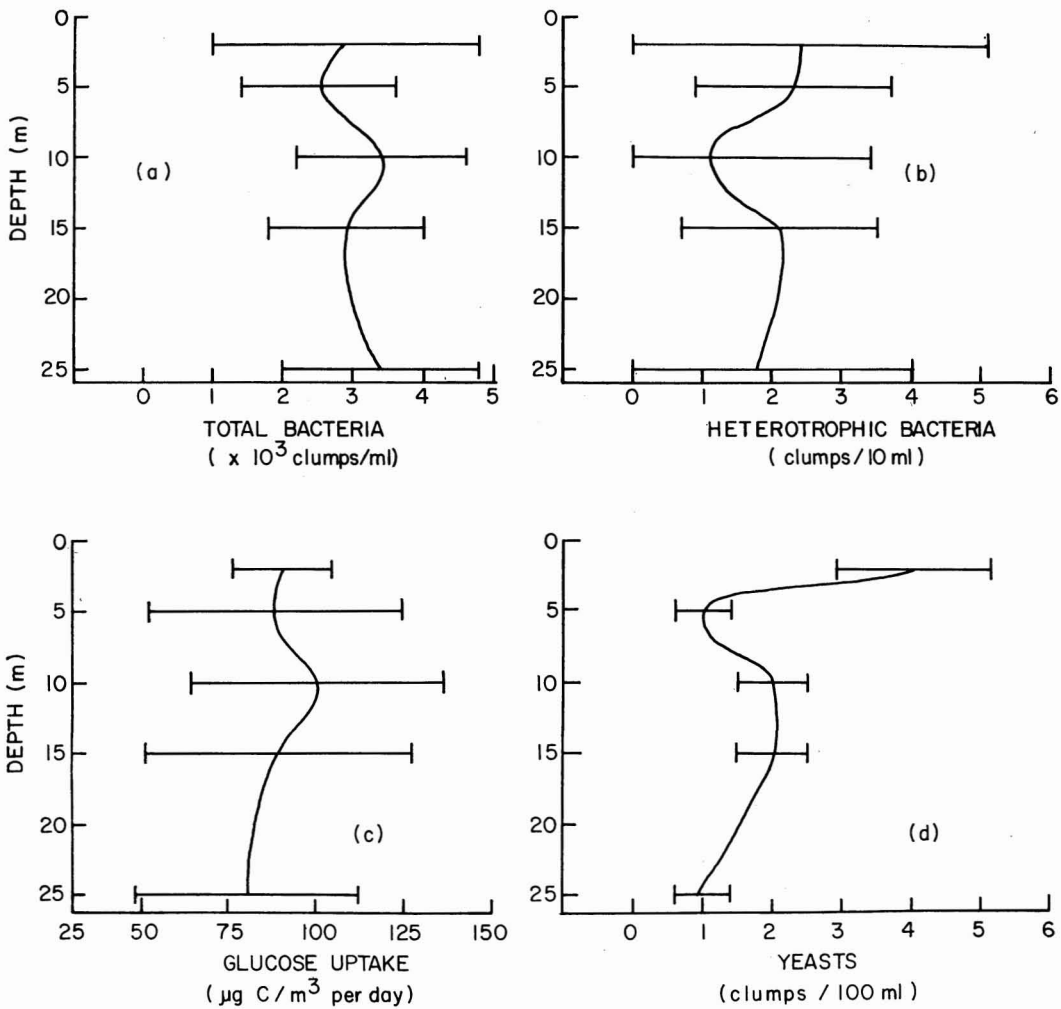


FIG. 2. Vertical distribution of total bacteria (a), heterotrophic bacteria (b), yeasts (c), and glucose uptake by microorganisms (d) in the North Pacific subarctic water. Horizontal bars indicate the standard deviation of the variance.

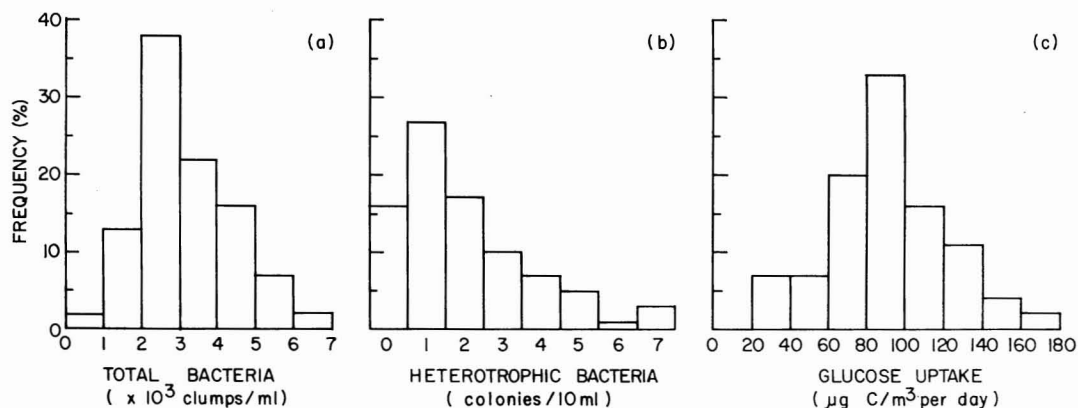


FIG. 3. Frequency distribution histogram of total bacteria (a), heterotrophic bacteria (b), and glucose uptake by microorganisms (c) in the euphotic zone of the North Pacific subarctic water.

Figure 3. The average concentration of total bacteria was calculated to be 3.0×10^3 clumps per ml, and 31 ± 14 percent of the clumps were microbial aggregates having a diameter of 5μ or more. Most of the aggregates about 5μ in diameter were composed of bacteria and plankton fragments.

On a few occasions bacterial colonies were also detected on the mucus of large aggregates of living phytoplankton up to 100μ in size. Therefore, on such particulate matter there were a large number of bacteria which could not be counted under the microscope. As particulate matter of 5μ diameter has a volume of approximately $60\mu^3$, and as an average bacterium has a volume of $0.5\mu^3$, approximately 100 bacterial cells would have been present in such aggregates (determined by the direct microscopic counts). The number of total bacteria was about 10^4 -fold greater than the number of heterotrophic bacteria. Pure cultures of heterotrophic bacteria, isolated from a depth of 10 meters at Stations 04, 07, and 12, took up approximately 10^{-9} μg carbon as glucose per cell per day under the same conditions as were employed for the field observations. On the other hand, about 90 μg of glucose-carbon were taken up by microorganisms per m^3 per day, which means that a microbial biomass equivalent to 10^5 cells of heterotrophic bacteria per ml may have been present in the water. Therefore, one bacterial clump is calculated to have had an average of 30 cells (by the physiological method), because 3.0×10^3 clumps of bacteria

were measured per ml. Using the Coulter counter by the method of Sheldon and Parsons (1967), the average cell volume of the yeasts in the sea was found to be about $100\mu^3$ (Seki and Kennedy, 1969). As the highest concentration of yeasts encountered in the ocean during this investigation was 8 clumps per 100 ml, and as only about 2 clumps per 100 ml was usually found, the biomass of yeasts was estimated to be less than 0.01 percent of the bacterial biomass.

DISCUSSION

During the cruise in the North Pacific subarctic water in spring, it was observed that the total bacteria were more abundant than were the heterotrophic bacteria in the euphotic zone, the ratio of clumps being ten thousand to one. This ratio is quite common in the pelagic region of the Pacific Ocean. The only marine fungi detected were yeasts, and their biomass was less than 0.01 percent of that of the heterotrophic microorganisms.

No significant change in the vertical distribution of microbial biomass was observed from the surface down to 25 meters, although there were microzonations in the distribution of each microorganism. Such microzonations in the euphotic zone must be largely influenced by processes of succession, from the phytoplankton blooms to increases in heterotrophic microorganisms, as was observed by Parsons and Seki (1969).

Marine bacteria and allied microorganisms

have been thought to play a most important part in the food chain of the pelagic region of the sea. Seki and Kennedy (1969) have confirmed that heterotrophic microorganisms are an important food source for zooplankton in the coastal region of the sea when the primary production was low. During the Transpacific Expedition, it was determined by two different methods (direct count and a physiological method) that the most probable bacterial biomass was approximately 10^5 cells per milliliter of seawater. This biomass corresponds to 50 mg wet bacteria per m^3 , which is 2 μg bacterial-carbon per liter. The chemical data obtained by the Pacific Oceanographic Group (e.g., McAllister, Parsons and Strickland, 1960) show that 100 to 200 μg of carbon as particulate matter, or around 20 μg of phytoplankton-carbon are contained per liter. Therefore, 10 percent of the phytoplankton biomass, or 1 percent of the particulate organic matter, is estimated to consist of marine bacteria and allied microorganisms.

Thus, using the table in Parsons and Seki (1969), it is calculated that the standing crop of heterotrophic microorganisms is too small for the maintenance of copepods. On the other hand, as the bacteria form clumps in the sea, they become more suitable as prey for the zooplankton as an auxiliary food source. In addition, for such bacteriovorous protozoa as those isolated by Lighthart (1969), they may play a significant role in the food chain in the ocean, as was shown in the laboratory model by Seki (1964).

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